中国认可
国际互认
检测
TESTING
CNAS L3940

Final Report

In vitro cytotoxicity test-MTT cytotoxicity test

B-S2025-090528E-51

Test Article: **Light-curing resin (surgical guides, trays)**

Sponsor:

Name: Shenzhen Zhongchuang Medical Group Co., Ltd.

Address: Room 204, Administration Building, Jia Tai Pharmaceutical, No.1 Industrial Zone, Tangwei Community, Fuhai Street, Bao'an District, Shenzhen City

Manufacturer:

Name: Shenzhen Zhongchuang Medical Group Co., Ltd.

Address: Room 204, Administration Building, Jia Tai Pharmaceutical, No.1 Industrial Zone, Tangwei Community, Fuhai Street, Bao'an District, Shenzhen City

四川医疗器械生物材料和制品检验中心有限公司 检验专用章

Sichuan Testing Center for Biomaterials and Medical Devices Co., Ltd.

Supplementary Explanation

1. The report is invalid without the special inspection seal of inspection body and laboratory and the signature of the approving person.
2. The report that has been altered, added or deleted is invalid. The copies of the report without the special seal for inspection are invalid.
3. If there is any objection to the report, it shall be submitted to inspection body and laboratory in writing within 15 days from the date of receiving the report.
4. The data and conclusions in this report are the inspection results of the items tested in the sample.
5. The results and name of the institution of the report shall not be used for advertising, evaluation and commercial publicity without the written approval.

TEST FACILITY:
Address: Biomaterials Building in Sichuan University 29 Wangjiang Road, Chengdu, P.R. China
Zip code: 610064
Tel: +86-28-85412428
Fax: +86-28-85412428
Website: www.safetest.com.cn
E-mail: Standard42@163.com

CONTENTS

Statement of Quality Assurance Activities

This study has been reviewed in accordance with the provisions of the ISO/IEC 17025:2017. Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the report results accurately reflect the raw data of the study.

Phase Inspected	Date inspected	Auditor	Date reported to Management
Test	2025-09-22	Tun Yuan	2025-09-22
Report generation	2025-10-24	Qiu Li	2025-10-24

Wen Zou

QA Representative: Wen Zou

2025-11-07

Date:

1. Summary

Based on the ISO 10993-5: 2009, an *in vitro* cytotoxicity study was conducted on the test articles to determine the potential for cytotoxicity of the test article. The cytotoxicity of the test article was evaluated according to cell viability percentage of MTT cytotoxicity test.

L929 cells were seeded into 96-well plates and maintained in culture for 24 hours (~1 doubling period) to form a semi-confluent monolayer. They were then exposed to the test compound over a range of concentrations. After 24 hours exposure, examine the cells microscopically, the formazan formation was determined for each treatment concentration and compared to that determined in control cultures. For each treatment the percentage inhibition of growth was calculated.

Under the conditions of the study, when the cells contacted with extract liquid for 24 hours, examine the cells microscopically, there were discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth of the 100% extract liquid concentration group, and the cell viability percentage of the 100% of the extract liquid concentration group was 87.4%. The negative controls and the positive controls met the requirements. Under the conditions of this study, the microscopic observation revealed that morphological grading of cytotoxicity of the 100% of the extract liquid of test article showed non-cytotoxicity, Grade 0 (qualitative evaluation); cell viability percentage of the 100% of the extract liquid concentration group was above 70% (quantitative evaluation).

Study and Supervisory

Personnel: **Yongfu Cai**
Xiaoqin Yang

Study supervisor: Qiu Li
Qiu Li

Director: Jie Liang 2025-11-07
Jie Liang **Date Completed**

This report is authorized by signatures above.

Sichuan Testing Center for Biomaterials and Medical Devices Co., Ltd.

2. Introduction

2.1 Purpose

The test was used to evaluate cytotoxicity of the test article *in vitro*.

2.2 Testing Guidelines

ISO 10993-5:2009 Biological evaluation of medical devices Part 5: Tests for *in vitro* cytotoxicity.
ISO 10993-12:2021 Biological evaluation of medical devices Part 12: Sample preparation and reference materials.

2.3 Dates

Test Article Received:	2025-09-15
Testing Started:	2025-09-22
Testing Ended:	2025-09-26

3. Compliance

Sichuan Testing Center for Biomaterials and Medical Devices Co., Ltd. has been accredited by China National Accreditation Service for Conformity Assessment (CNASL3940).

4. Materials

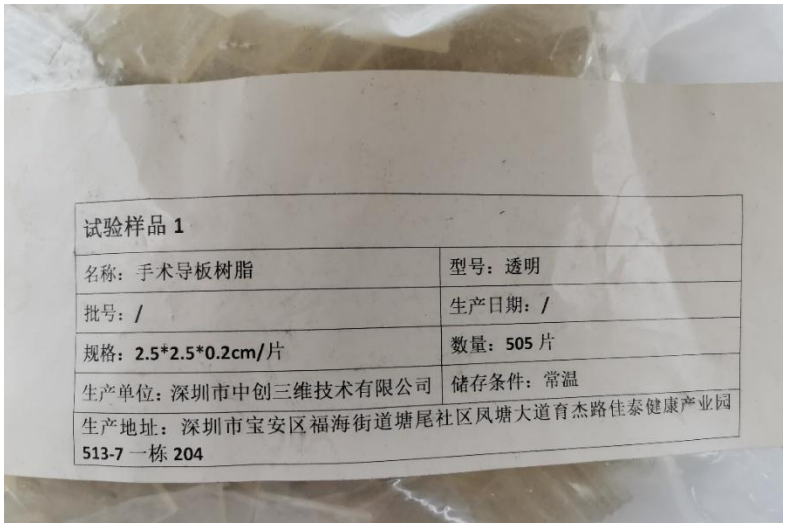
4.1 Test sample

Test Article:	Light-curing resin (surgical guides, trays) (Fig.1)
Sample Serial No.:	S2025-090528
Specification:	2.5*2.5*0.2cm/unit
Model:	Transparent; blue
Batch:	/
Physical State:	See test article image
Storage Conditions:	Room temperature

The above test sample information is provided by the sponsor. The sponsor is responsible for all test article characterization data.


S2025-090528

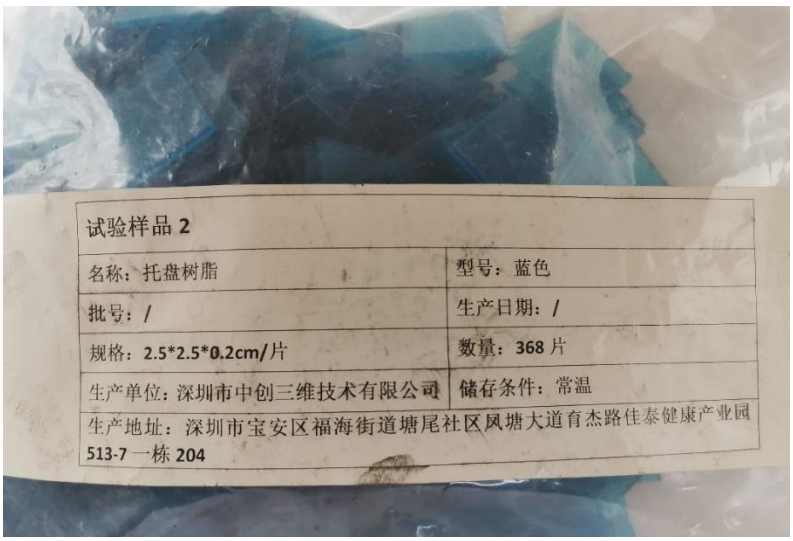
A


试验样品 1
名称：手术导板树脂
型号：透明
批号：/
生产日期：/
规格：2.5*2.5*0.2cm/片
数量：505 片
生产单位：深圳市中创三维技术有限公司
储存条件：常温
生产地址：深圳市宝安区福海街道塘尾社区凤塘大道育杰路佳泰健康产业园 513-7 一栋 204

B


S2025-090528

C



D

Fig.1. Photograph of the sample. A. The test sample for use 1; B. Sample label 1; C. The test sample for use 2; D. Sample label 2.

4.2 Negative Control

Name:	HDPE
Manufacturer:	Shandong Usolf Chemical Technology Co., Ltd.
Specification:	500g
Batch:	089998007200
Colour:	White
Physical State:	Solid
Storage Conditions:	Room temperature.

4.3 Positive Control

Name:	DMSO
manufacturer:	MP Biomedicals
Specification:	100mL
Batch:	YC0403
Colour:	Colourless
Physical State:	Liquid
Storage Conditions:	Room temperature

4.4 Blank Control

Name:	MEM (containing 10% FBS)
Preparation date:	2025-09-22
Colour:	Pink
Physical State:	Liquid
Storage Conditions:	2℃-8℃

4.5 Reagent

MEM medium	gibco, Lot: 6124570
Fetal Bovine Serum	Every Green, Biology, Lot: 23060701
Penicillin – Streptomycin Solution	gibco, Lot: 234137
L-Glutamine	Gibco, Lot: 3023586
MTT	Sigma-Aldrich, Lot: P2027676

5. Identification of test system

Cells: The test system was mouse fibroblast L929 cells (NCTC Clone 929, L cell, L-929, derivative of Strain L).
Source: The cells were purchased from the cell bank of the committee for the conservation of typical cultures, Chinese academy of sciences, December 02, 2020.

6. Justification of test system

The mouse fibroblast L929 cells have been used for cytotoxicity studies because they have been demonstrated sensitivity to extractable cytotoxic articles.

7. Test system management

Personnel: Associates involved were appropriately qualified and trained.
Operation: Sterile operation was used during culture.
Environmental: The room temperature was monitored daily. The room temperature range was 18℃-24℃. The room humidity was monitored daily. The humidity range was 40%-70%.

8. Test design

8.1 Sample preparation

Test Article: 2h ultraviolet irradiation was used for the test article sterilization. The test article was prepared aseptically. According to the sponsor's requirements, the transparent sample and the blue sample were mixed in 1:1. The test article extract liquid was prepared as shown in Table 1. The extracts were tested immediately following extraction. The extracts were not centrifuged, filtered, or otherwise altered prior to using.

Blank: Extract media without sample was prepared as shown in Table 1.

Negative Control: HDPE was sterilized by 121℃ for 20min and prepared as shown in Table 1

Positive Control: 10% DMSO was included in 90% extract media.

Extract media: 88%MEM medium, 10% Fetal Bovine Serum, 1% Penicillin – Streptomycin Solution, 1% L-Glutamine Solution.

Table1. Extracts preparation information

	Extraction Ratio	Amount	Volume of Vehicle	Extraction Condition
Test Article	3cm^2/mL	58.0cm^2	19.3mL	37℃, 60rpm, 72h
Blank	/	/	20.0mL	37℃, 60rpm, 72h
Negative Control	0.2g/mL	2.1g	10.5mL	37℃, 60rpm, 72h
Positive Control	/	1.0mL	9.0mL	/

Table2. The condition of extracts

Extract	Condition of Extracts		
	Color	Clarity	Particulates
Test	Pink	Clear	None
Blank	Pink	Clear	None
Negative Control	Pink	Clear	None
Positive Control	Pink	Clear	None

8.2 Preparation of cells:

Mouse fibroblast cells (NCTC Clone 929, L cell, L-929, derivative of Strain L) were proliferated at 37℃, 5% CO_2 in petri dishes (Φ100mm) containing MEM supplemented with 10% fetal bovine serum and antibiotics (100U/mL penicillin, 100μg/mL streptomycin). After grew nearly confluent, the cells were trypsinized, collected and adjusted to 1×10^5 cells/mL for following experiment.

8.3 Test procedure

Stirred cell suspension was pipetted into 96-well plate with 1×10^4 cells/well, incubated at 37℃, 5% CO_2 for 24 hours. Then the liquid was discarded, and 100μL of the extract liquid (containing 10% FBS) with different dosage was added into each well with 6 parallel wells (row 3~6; other 6 wells adding MEM (containing 10% FBS) were used as blank control (row 2 and row 11), the extract liquid of HDPE as negative control (row 7) and 6 wells with 10% DMSO (ultimate concentration) as positive control (row 9). After cultured at 37℃, 5% CO_2 for 24 hours, the liquid was discarded, and 50μL MTT solution (1mg/mL, dissolved in MEM medium) was added into each well and incubated another 2 hours.

At the end of incubation, the liquid in the 96-well was discarded, 100μL of isopropanol was added and vortexed to allow total color released from the bottom of 96-well plate, the absorbency was measured at 570nm by a microplate reader (reference wavelength 650nm). The cell viability percentage was calculated by comparing with the blanks (The cell viability percentage of the blanks represented as 100%).

9. Evaluation of results

Qualitative evaluation: Examine the cells microscopically to assess the conditions of all the cultured cells by table3. The achievement of a numerical grade greater than 2 was considered a cytotoxic effect.

Table3. Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50 % of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50 % growth inhibition observable.
3	Moderate	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not completly destroyed, but more than 50 % growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Quantitative evaluation: According to the measured data, the average OD value of each group was calculated, and according to the formula. Reduction of cell viability by more than 30 % was considered a cytotoxic effect.

$$\text{Viab.\%} = \frac{100 \times OD_{570e}}{OD_{570b}}$$

Where:

OD_{570e} is the mean value of the measured optical density of the 100 % extracts of the test sample;

OD_{570b} is the mean value of the measured optical density of the blanks.

10. Results

Qualitative evaluation: The results of the test were shown in Fig.2. Examine the cells microscopically, there were discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth of the 100% extract liquid concentration group. The results proved that morphological grading of cytotoxicity of 100% of the extract liquid of test article showed non-cytotoxicity, Grade 0.

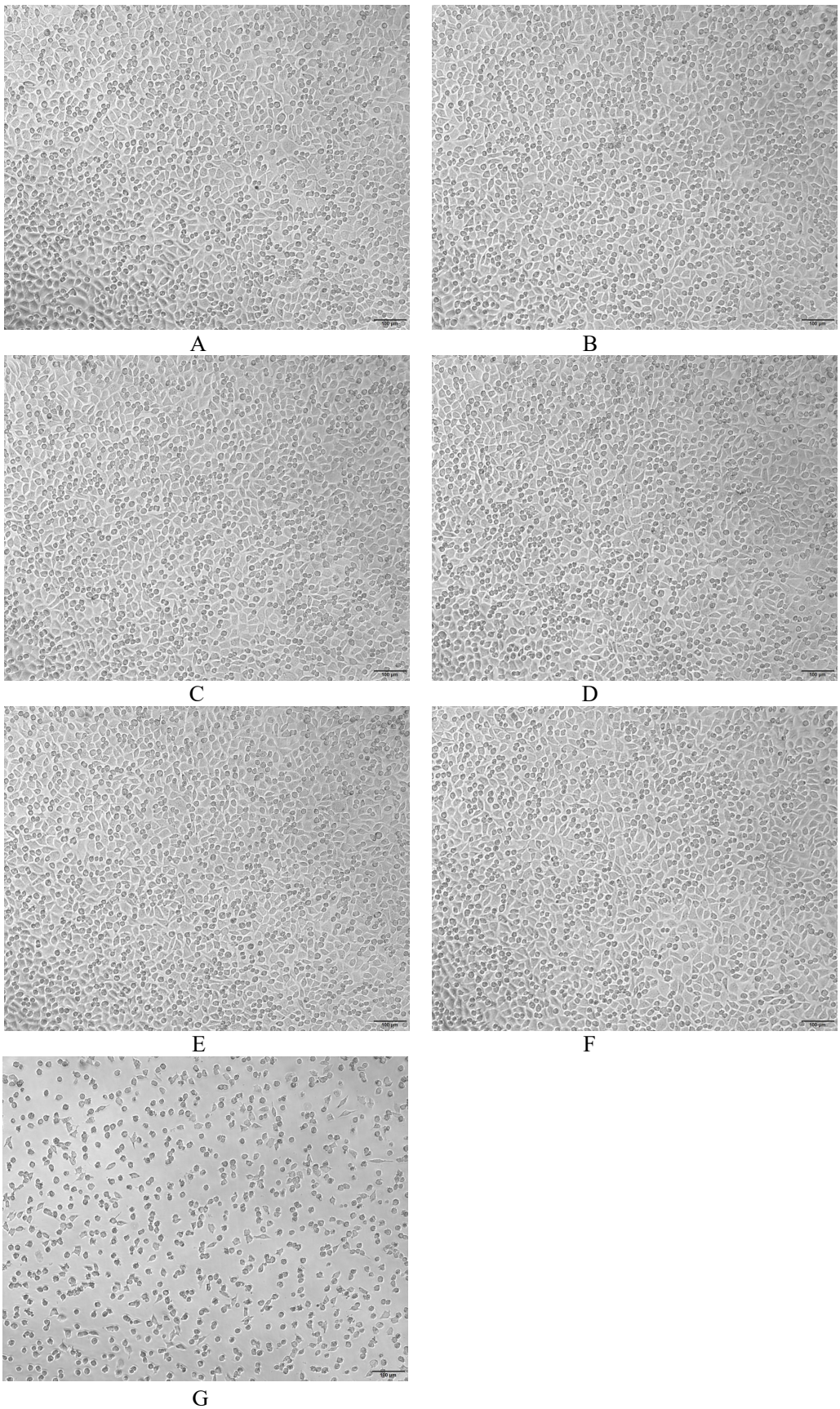


Fig.2. the cells were cultured in different extract liquid concentration and different control sample. A. 100% of the extract liquid concentration group; B. 50% of the extract liquid concentration group; C. 25% of the extract liquid concentration group; D. 12.5% of the extract liquid concentration group; E. Negative control group; F. Blank control group; G. Positive control group.

Quantitative evaluation: The results of the test were shown in table4. Under the conditions of the study, when the cells contacted with extract liquid for 24 hours, cell viability percentage of the 100%, 50%, 25% and 12.5% of the extract liquid concentration groups were 87.4%, 90.0%, 94.0% and 97.1% respectively, while cell viability percentage of the positive control group and the negative control group were 8.5% and 98.3%. The negative controls and the positive controls met the requirements. Cell viability percentage of the 100% of the extract liquid concentration group was above 70%.

Table4. The results of optical density in the MTT cytotoxicity test

	Sample group				Negative	Blank		Positive
	100%	50%	25%	12.5%		1	2	
Well 1	0.949	1.020	1.046	1.118	1.106	1.158	1.156	0.099
Well 2	0.993	1.018	1.061	1.097	1.118	1.154	1.108	0.085
Well 3	0.998	1.006	1.068	1.106	1.120	1.145	1.123	0.092
Well 4	0.986	1.021	1.062	1.089	1.097	1.138	1.149	0.094
Well 5	0.978	1.000	1.049	1.061	1.116	1.129	1.041	0.096
Well 6	0.988	1.008	1.055	1.076	1.074	1.145	1.040	0.105
Average OD	0.982	1.012	1.057	1.091	1.105	1.124		0.095
Viab. %	87.4%	90.0%	94.0%	97.1%	98.3%	100.0%		8.5%

Results apply only to the test article tested. No further evaluation of these results is made by. Any extrapolation of these data to other samples is the responsibility of the sponsor.

11. Conclusion

Under the conditions of this study, the microscopic observation revealed that morphological grading of cytotoxicity of the 100% of the extract liquid of test article showed non-cytotoxicity, Grade 0 (qualitative evaluation); cell viability percentage of the 100% of the extract liquid concentration group was above 70% (quantitative evaluation).

12. Quality assurance

The study has been performed according to standard operating procedures and standard protocols. Inspections were conducted at intervals adequate to assure the integrity of the study in compliance with ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. The final report was reviewed for conformance to the section 7.8 of ISO/IEC 17025: 2017.

13. Deviation

This experiment was conducted according to ISO 10993-5:2009 Biological evaluation of medical devices Part 5: Tests for *in vitro* cytotoxicity.

14. Record storage

All raw data pertaining to this study and a copy of the final report are to be retained in designated archive files in our inspection body and laboratory.